



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number : **0 641 790 A1**

(12)

EUROPEAN PATENT APPLICATION

(21) Application number : **94306486.5**

(51) Int. Cl.⁶ : **C07D 311/72, A61K 31/355**

(22) Date of filing : **02.09.94**

(30) Priority : **03.09.93 GB 9318271**

(43) Date of publication of application :
08.03.95 Bulletin 95/10

(84) Designated Contracting States :
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC
NL PT SE**

(71) Applicant : **SCOTIA HOLDINGS PLC**
Efamol House
Woodbridge Meadows
Guildford Surrey GU1 1BA (GB)

(72) Inventor : **Horrobin, David F., Dr.**
Scotia Phar. Ltd.,
Efamol House Woodbridge Meadows
Guildford, Surrey GU1 1BA (GB)
Inventor : **Manku, Mehar S., Dr., Scotia**
Pharmaceuticals Ltd.
Research & Development Centre,
Kingstown Broadway
Kingstown Ind. Estate, Carlisle CA3 0HA (GB)

(74) Representative : **Sturt, Clifford Mark et al**
J. MILLER & CO.
34 Bedford Row
Holborn
London WC1R 4JH (GB)

(54) **Tocopherols.**

(57) A natural vitamin E preparation, i.e. vitamin E extracted from natural sources, comprising one or more of the alpha-, beta-, gamma- and delta-tocopherol forms, either as the tocopherol itself, or as a derivative, such as the acetate, in which the total concentration of Wendt PAHs is less than 100 ppb.

EP 0 641 790 A1

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds which are near ubiquitous in the environment. Some of these compounds are carcinogenic. They are produced in the course of many different types of industrial activity and are present in substantial amounts in the atmosphere. They are picked up by plants from the atmosphere and so, paradoxically, some of the foods which are regarded as healthy are the most contaminated.

Wendt described the major PAHs likely to be present in foodstuffs and identified in particular 6 "light" and 7 "heavy" PAHs (see Table 1). The heavy PAHs are the ones which are most likely to be toxic.

We produce vegetable oils and are concerned to ensure that PAH levels in products for sale are kept well within appropriate levels. Recently what is regarded as an unacceptable level of PAHs was found in a finished product to which had been added natural vitamin E. The PAH levels in the oil itself were well within acceptable limits. After a great deal of investigation of all possible sources of PAH contamination it was concluded that the excess PAH had been added in the natural vitamin E. This was surprising, since we had not previously known of any reports of PAH contamination of vitamin E. However, natural vitamin E comes from plant sources, usually soya or wheat, and is prepared by a series of concentration steps which could well concentrate the PAHs as well as the vitamin E.

There are three major manufacturers of natural vitamin E in the world, and samples were obtained from all three. The samples were analysed for the 13 PAHs in the Wendt classification. The results are shown in Table 1 below. The Table sets out the 13 PAHs identified by Wendt as possible important contaminants of foods and their levels in commercial samples of d-alpha-tocopherol acetate supplied by three manufacturers. All figures are in ppb.

TABLE 1

Light PAHs	Supplier A	Supplier B	Supplier C
Phenanthrene	27	<1	<2
Anthracene	4	59	125
Fluoranthene	4	106	34
Pyrene	4	3244	2018
Benzo(a)anthracene	2	9	42
Chrysene + triphenylene	10	191	2328
Heavy PAHs			
Benzo(a)pyrene	28	30	106
Benzo(e)pyrene	285	2	521
Perylene	35	2	289
Anthanthrene	10	<1	4
Benzo(g,h,i)perylene	81	2	90
Dibenzo(a,h)anthracene	5	<1	<1
Coronene	1	<1	2
Total	526	3646	5560

As can be seen from Table 1, while the samples vary, they all contain unacceptably high levels of one or more PAHs. Thus natural vitamin E preparations, either used by themselves or added to other foods or food supplements, could prove to be significant sources of human PAH intake.

The final end use of the vitamin E is clearly relevant in the determination of satisfactory PAH levels. The invention provides a natural vitamin E preparation comprising one or more of the alpha-, beta-, gamma- and delta-tocopherol forms, either as the tocopherol itself, or as a derivative, such as acetate, in which the total

concentration of Wendt PAHs is less than 100 ppb. The PAH concentration in the preparation is further suitably less than 60 µg/kg (ppb), more suitably less than 30 µg/kg (ppb), preferably less than 15 µg/kg (ppb), more preferably less than 5 µg/kg (ppb) and most preferably less than 1 µg/kg (ppb). Obviously the more natural vitamin E is incorporated in any end use, the lower should be the permitted concentration.

5 The vitamin E preparation may be used by itself as a nutritional supplement or as an agent to be added to other foods, food additives, nutritional supplements, pharmaceuticals, skin care products or products of any other type for human or veterinary use. Ordinarily the required PAH levels will be reached by purification, but we do not exclude the case when the vitamin E is from sources selected so as to have acceptable PAH levels, naturally or by control of the growing environment.

10 Various techniques are suitable for the removal of PAHs from natural lipids. In general, the following techniques may all be used to produce oils and vitamin E preparations with satisfactory PAH levels. These methods include but are not limited to the following:

1. Liquid/liquid extraction, using for example mixer-settlers, countercurrent extractors, centrifugal extractors or columns. The principle is to mix the tocopherols with a light non-polar solvent such as cyclohexane, hexane or other appropriate hydrocarbon. This mixture is then mixed with any appropriate polar solvent such as dimethylformamide (DMF)/water, or sulfolane/water. The PAHs are extracted into the polar solvent fraction which is then separated from the tocopherol fraction by standard industrial techniques and, if appropriate, further purification can be achieved by passing through a silica gel column.
- 15 2. High pressure liquid chromatography (HPLC) using any appropriate commercially available system can be used. Typical column packings for straight phase are LiChrosorb Si 60 or equivalent, or for reverse phase separation a C18 column with 30-40 µm packing. For normal phase operation an appropriate isocratic condition solvent is iso-octane:isopropanol 99.5:0.5 v/v or equivalent. For reverse phase operation with the tocopherol material dissolved in a solvent such as acetone, a suitable solvent system is methanol:water, 99.5:0.5 v/v or equivalent.
- 20 3. Flash chromatography using a nitrogen or other appropriate gas driven column. As an example, a glass column can be packed with Silica Gel 60, a prefilter containing charcoal such as Norit SA4 and a filter such as celite. The contaminated tocopherols are dissolved in hexane at, for example, a 1:2 ratio and become separated when passing through the column and are collected. The solvent is then removed to produce the purified tocopherols. Alternatively, ultrapurification of tocopherols can be achieved by flash chromatography using a C18 type packing.
- 30 4. Methods using formation of complexes between caffeine and PAHs with separation of the non-complexed tocopherols.
5. Supercritical fluid extraction followed by preparative or flash column chromatography.
- 35 6. Treatment of natural tocopherol mixtures with sulphuric acid or like agent followed by extraction with hexane or other appropriate solvent and final purification by flash chromatography.

Examples of methods for reducing the PAH levels in vitamin E

A. Liquid-liquid extraction methods

40 There are several ways of doing this, some of which are set out below:

1. Mix the tocopherol (TP) with 10 times the volume of cyclohexane, pestiscan grade. Add to this an approximately equal volume of a 90:10 mixture of dimethylformamide:water and mix thoroughly for about 2 minutes. Allow the mixtures to settle and separate. The lower aqueous phase containing the PAHs is then removed. The upper cyclohexane layer containing the TP is then washed again in the same way with a dimethylformamide:water mixture. The upper cyclohexane layer is then collected and evaporated in a rotary evaporator under high vacuum to distill off the cyclohexane leaving behind the PAH-free TP.
- 45 2. Mix the TP with 10 times the volume of cyclohexane, pestiscan grade. Add an approximately equal volume of a 90:10 sulfolane:water mixture and mix thoroughly for about 3 minutes. Allow the mixtures to settle and separate and remove the lower aqueous layer containing the PAHs. Wash the upper cyclohexane layer again with the sulfolane:water mixture. Collect the upper cyclohexane layer and evaporate under high vacuum in a rotary evaporator. The material left after solvent removal is the PAH-free TP.
- 50

B. Slurry technique

55 Prepare a Celite filter pad in a funnel of appropriate size using Celite 521 filter agent (BN374020114 from Fisons) or a similar material. Add 0.5% by weight of charcoal (such as Norit SA4) to the TP. Mix the TP and charcoal well and filter the slurry through the Celite filter using a vacuum. The filtrate is the PAH-free TP.

C. Column chromatography

This can be done using a number of different columns, solid phases and solvents. For example, a column can be loaded with silica gel 60 (6 length units), charcoal (Norit SA4) (7 length units) and Celite 521 (2 length units). The TP is mixed with about twice its volume of HPLC grade hexane and applied to the column under nitrogen pressure. The column is then eluted with more hexane and the eluent collected. The hexane is then evaporated under vacuum leaving behind the PAH-free TP.

D. Caffeine-formic acid complexation

TP is dissolved in about five times the volume of cyclohexane, pestiscan grade. A volume of formic acid/caffeine solution equal to the original volume of TP is then added and mixed. The formic acid/caffeine solution is made by diluting 9 parts of 98-99% formic acid with 1 part of HPLC grade water, in which is dissolved 15% by weight of caffeine.

After mixing the lower formic acid/caffeine layer was discarded and the cyclohexane layer washed again with a further quantity of formic acid/caffeine. The cyclohexane layer was collected and evaporated under high vacuum to remove excess solvent, leaving behind the PAH-free TP.

Results of tests to reduced PAHs in commercial vitamin E samples

A commercial sample of vitamin E was tested for the presence of the Wendt PAHs. The six light PAHs are not considered to be harmful to health in any important way: they are phenanthrene, anthracene, fluoroanthrene, pyrene, benzo(a)anthracene, chrysene and triphenylene. The seven heavy PAHs on the other hand are regarded as potentially dangerous, particularly with regard to carcinogenicity. They are:

benzo(e)pyrene, benzo(a)pyrene, perylene, DiB(a,h)anthracene, benzo(ghi)perylene, anthanthrene and coronene.

Natural vitamin E was purchased from a commercial supplier and analysed for the light and heavy PAHs. The sum of the light PAHs in the sample was 25.7 microg/kg whereas the sum of the heavy PAHs was 299.4 microg/g.

The natural vitamin E was then subjected to treatment by four of the five methods set out in the examples. The levels of PAHs in the vitamin E after treatment are shown below:

Treatment	Heavy PAHs	Light PAHs
Cyclohexane/dimethylf.	12.8	6.3
Cyclohexane/sulfolane	5.6	11.3
Column chromatography	16.8	10.5
Caffeine/formic acid	30.4	14.4
All figures are in microg/g.		

It can be seen that all the techniques produced a very substantial reduction in the PAH levels. This is particularly evident for the heavy PAHs which are brought down to values which are regarded as safe for human consumption. The light PAHs, which are not regarded as dangerous, are also substantially reduced.

Claims

1. A natural vitamin E preparation, i.e. vitamin E extracted from natural sources, comprising one or more of the alpha-, beta-, gamma- and delta-tocopherol forms, either as the tocopherol itself, or as a derivative, such as the acetate, in which the total concentration of Wendt PAHs is less than 100 ppb.
2. A preparation according to claim 1, wherein the total concentration is less than 60 ppb.
3. A preparation according to claim 2, wherein the total concentration is less than 30 ppb.
4. A preparation according to claim 3, wherein the total concentration is less than 15 ppb.

5. A preparation according to claim 4, wherein the total concentration is less than 5 ppb.
6. A preparation according to claim 5, wherein the total concentration is less than 1 ppb.
- 5 7. A natural vitamin E preparation according to any preceding claim, wherein the PAH concentration has been reached by purification of the vitamin E with selective removal of PAH.
8. A preparation according to any preceding claim for use as a nutritional supplement or as an agent to be added to other foods, food additives, nutritional supplements, pharmaceuticals, skin care products or products of any other type for human or veterinary use.

10

15

20

25

30

35

40

45

50

55



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 6486

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	CHEMICAL ABSTRACTS, vol. 94, no. 13, 1981, Columbus, Ohio, US; abstract no. 103165c, page 724 ; * abstract *	1,7	C07D311/72 A61K31/355
X	& JP-A-8 081 879 (NSSHIN FLOUR MILLING) 16 December 1978 ---	1,7	
X	CHEMICAL ABSTRACTS, vol. 105, no. 30, 1986, Columbus, Ohio, US; abstract no. 134192j, page 703 ; * abstract *	1,7	
X	& JP-A-60 149 582 (MITSUI PETROCHEMICAL INDUSTR.) 7 August 1985 ---	1,7	
X	CHEMICAL ABSTRACTS, vol. 108, no. 17, 1988, Columbus, Ohio, US; abstract no. 203390y, BILIC, N. ET AL. 'USE OF A COLUMN FOR EXTRACTION DRYING AND PURIFICATION OF RETINOL AND TOCOPHEROL BY HPLC' page 565 ; * abstract * & FRESENIUS Z. ANAL. CHEM., vol. 330, no. 2, 1988, SWITZ. pages 143 - 145 ---	1,7	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07D
X	FR-A-1 360 759 (KODAK) * page 1 - page 8 * ---	1,7	
X	FR-A-2 602 772 (RHONE-POULENC) * page 1 - page 7 * ---	1,7	
X	EP-A-0 171 009 (HENKEL CORPORATION) * the whole document * ---	1,7,8	
	--- -/--		
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 10 November 1994	Examiner Francois, J
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application I : document cited for other reasons --- & : member of the same patent family, corresponding document	

EPO FORM 1501 (01/94) (P.O. 01/94)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 6486

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EP-A-0 421 419 (BRISTOL-MYERS SQUIBB CY) * page 18 - page 27; claims * -----	1,7,8	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 10 November 1994	Examiner Francois, J
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

EPO FORM 1503 (01/91) (P0404)